

Recognition of *Yersinia enterocolitica* multiple strain infection in twin infants using PCR-based DNA fingerprinting

J.T. Gray¹, M. WaKabongo², F.E. Campos³, A.A. Diallo⁴, C. Tyndal³ and C.A. Tucker⁵

¹USDA, ARS, Antimicrobial Resistance Research Unit, Athens, GA, ²Department of Microbiology and Immunology, University of Osteopathic Medicine and Health Science, Des Moines, IA, ³Children's and King's Daughters Hospital, Norfolk, VA, ⁴Public Health Department, Norfolk, VA and ⁵Des Moines General Hospital, IA, USA

486/7/00: received 31 July 2000, revised 30 October 2000 and accepted 7 November 2000

J.T. GRAY, M. WAKABONGO, F.E. CAMPOS, A.A. DIALLO, C. TYNDAL AND C.A. TUCKER. 2001.

Aims: *Yersinia enterocolitica* causes several syndromes in humans. The most common presentation is enterocolitis in children, presenting as fever and diarrhoea. A *Y. enterocolitica* multiple strain infection in twin infants was investigated.

Methods and Results: One isolate was recovered from one patient and two morphologically-different isolates were recovered from the other infant. Biochemically, all isolates were identified as *Y. enterocolitica* group. The genomic DNA from each strain was purified and DNA fingerprinting was performed. The banding patterns observed for *Y. enterocolitica* isolates 2 and 3, from patients 1 and 2, respectively, were identical when comparing the presence or absence of major bands. However, *Y. enterocolitica* isolate 1, from patient 1, showed a distinctive banding pattern from isolates 2 and 3.

Conclusions: The findings indicate that one infant was colonized by more than one strain of *Y. enterocolitica*, demonstrating that multiple strains can colonize and invade a patient.

Significance and Impact of the Study: Recognition of multiple strain infections can be important in diagnosis, treatment and prognosis of *Y. enterocolitica* infections, as well as in disease epidemiology. The technique described here offers a straightforward method for strain comparison.

INTRODUCTION

Yersinia enterocolitica is a Gram-negative coccobacillus found in water and many animals such as rodents, rabbits, pigs, sheep, cattle, horses and domestic pets. Pigs serve as a principal reservoir for human pathogenic strains of serotypes O:3 and O:9 (Bottone 1997). The organism can be transmitted to humans through contaminated milk, water and other foods. The clinical presentations are wide in range and can include gastroenteritis, bacteremia and sepsis, septicemia, pharyngitis, enteritis, enterocolitis, acute mesenteric lymphadenitis and terminal ileitis. Most cases of gastroenteritis occur among infants and children (AlMohsen *et al.* 1997; Bottone 1997). Recently, the Center for Disease Control and Prevention has reported cases of *Y. enterocolitica* caused by

contaminated red blood cell transfusions (Centers for Disease Control and Prevention. 1997). *Yersinia enterocolitica* has been implicated in several cases of food-borne disease outbreaks in the United States (Bottone 1997).

The most common presentation of *Y. enterocolitica* infection in children is enteritis and enterocolitis (Kohl 1979; Lee *et al.* 1991; Janda and Abbot 1994). However, invasive *Y. enterocolitica* infection often results in bacteraemia in young children. Sepsis is usually associated with identified risk factors such as iron overload syndromes (AlMohsen *et al.* 1997). *Yersinia enterocolitica* is emerging as an important enteric pathogen, particularly among black children in the USA (Lee *et al.* 1991). One surveillance study documented 106 cases of *Y. enterocolitica* with 75.5% of patients being black and 70% of patients being less than 12 months old (McDonald *et al.* 1998). The study also found bacteraemia to be a common complication in children under 5 months of age. Several

Correspondence to: J.T. Gray, USDA, ARS, ARRU, 950 College Station Road, Athens, GA 30605, USA (e-mail: jgray@saa.ars.usda.gov).

reports have discussed the clinical features and appropriate treatments for children (AlMohsen *et al.* 1997; Bottone 1997).

The relative frequency of infection and the existence of many potential reservoirs amplifies the need for molecular epidemiologic tools and methods of strain comparison.

Epidemiological surveys of *Y. enterocolitica* have utilized a wide range of typing methods. At least one study has demonstrated differences in *Y. enterocolitica* strains that have been isolated from successive samplings from a single patient (Odinot *et al.* 1995). These types of data have led to the question as to whether these individuals have been colonized with more than one strain or whether the differences are due to minor genetic changes *in vivo* (Odinot *et al.* 1995). Typing methods available range from those of moderate discriminatory ability, such as biotyping (Wauters *et al.* 1987), serotyping, phage typing (Baker and Farmer 1982) and antibiogram typing (Odinot *et al.* 1995), to molecular techniques, such as restriction fragment length polymorphisms (RFLP) of rRNA genes (Nesbakken *et al.* 1987; Blumberg *et al.* 1991) and restriction endonuclease analysis of chromosomal DNA (Kapperud *et al.* 1990).

Arbitrarily-primed polymerase chain reaction (PCR) is a technique used to amplify DNA for detecting genomic polymorphisms. Low stringency conditions generate a reproducible array of strain-specific products. This allows differentiation between two closely-related isolates that may not have been possible through other methods (Mathews 1993; Welsh and McClelland 1993). There are many applications of random arbitrarily-primed PCR (RAPD PCR) because the method is generally fast and reliable. The technique has been used previously on a wide variety of pathogens, including *Acinetobacter*, *Aeromonas*, *Yersinia* and *Listeria* (Czajka *et al.* 1993; Grundman *et al.* 1997; Gray *et al.* 1998; Czajka and Batt 1994).

Using standardized reagents, the RAPD technique has been adapted for *Y. enterocolitica* in this study.

MATERIALS AND METHODS

Patient 1

The patient was admitted to the hospital with a 24 h history of fever and poor appetite. Her birth weight was 1.43 kg and she was hospitalized for 8 days following birth. She had recently been diagnosed with oesophageal reflux and was treated with cisapride and ranitidine. There was no history of diarrhoea; on the contrary, Karo syrup was given once because of constipation. Upon admission, she was described as a small infant, taking the bottle reluctantly, with a temperature of 39.2°C, respiration rate of 80 breaths min⁻¹ and heart rate of 190 beats min⁻¹. Her

physical examination was otherwise unremarkable, without a source for fever.

Patient 2

Patient 2 was admitted 1 day prior to the discharge of her twin sibling. She had a 24 h history of fever, and diarrhoea described as eight yellowish, loose stools. She had a normal appetite and had no reported vomiting. Her birth weight was 2.27 kg and she was reported to have had a fever of 37.7°C for 2 days. Upon admission, she had a fever of 38.1°C but this increased to 39.6°C later on the day of admission. Her respiration rate was 44 breaths min⁻¹ and her heart rate was 181 beats min⁻¹. Her physical examination was otherwise unremarkable, without any signs of toxicity or dehydration.

The isolates

Yersinia enterocolitica was isolated from both infants. Two morphologically-different isolates were recovered from patient 1 and designated isolates 1 and 2. One isolate was recovered from patient 2 and designated isolate 3. Complete biochemical phenotyping and antimicrobial susceptibility testing were performed.

Biochemical identification and susceptibility testing

Identification and susceptibility testing was performed using the MicroScan Data System and WalkAway System software (Dade International Inc., West Sacramento, CA, USA). The MicroScan Gram Negative Dried MIC/Combo Panel Identification System uses combination trays that provide biochemical identification and determine susceptibility patterns. The concentrations of antimicrobials cover the range of clinical interest. The breakpoint concentrations used were equal to the categorical breakpoints of the National Committee for Clinical Laboratory Standards (National Committee for Clinical Laboratory Standards (NCCLS) 1998).

Genomic DNA purification

Genomic DNA from *Y. enterocolitica* was isolated using the Wizard genomic DNA isolation kit (Promega, Madison, WI, USA). All *Y. enterocolitica* genomic DNA samples were diluted 1:25 in TE buffer (10 mmol l⁻¹ Tris, 1 mmol l⁻¹ EDTA, pH 7.4). Protein and nucleic acid concentrations were obtained using spectrophotometry at wavelengths of 260 and 280 using the Warburg Christian formula. All samples were then standardized to 10 ng µl⁻¹ of DNA.

Polymerase chain reaction

Genomic fingerprinting using standardized reagents was performed using the Pharmacia Ready-To-Go RAPD Analysis Beads (Pharmacia, Piscata Way, NJ, USA) for PCR. The RAPD Analysis Bead containing AmpliTaq DNA polymerase, Stoffel fragment, buffer and nucleotides was placed in a microcentrifuge tube. To each bead, 25 pmol l⁻¹ of primer were added and then 50 ng of purified template DNA. Deionized water (15 µl) was added and the bead was dissolved by vortexing. The 25 µl samples were placed in a Perkin Elmer 2400 thermocycler (Norwalk, CT, USA). A thermocycle of 95°C for 5 min, 1 cycle and 45 cycles of 95°C for 1 min, 36°C for 1 min and 72°C for 2 min was used. Samples were then stored at 4°C until agarose gel electrophoresis could be performed.

Visualization

Agarose gels (2%) were loaded with 5 µl of amplified DNA and 1 µl of loading dye. The gels were run at 125 V for approximately 1 h. Gels were placed in ethidium bromide and destained in water. Ultraviolet light was used for visualization of the bands.

Primer selection

In order to determine which primers would show distinct banding patterns between unique strains of *Y. enterocolitica*, six primers were tested in the development of the assay. Two primers were identified which created unique banding patterns, and these were used to compare the case study isolates. The primers which produced the most valuable banding patterns were primer 1 (GGTGCGGGAA) and primer 4 (AAGAGCCCGT).

RESULTS

Patients

Each of the infants, in this case, was diagnosed with invasive yersiniosis; however, both recovered without complication. Two morphologically-distinct *Y. enterocolitica* isolates were recovered from patient 1. Upon interview, the infants' uncle admitted to having had a recent one day history of watery diarrhoea and indicated that he had eaten chitterlings at a relative's house during the holidays. As the uncle routinely assists in the preparation of formula for the infants, this was viewed as the most likely source of the infection. However, a definitive source was not identified. Laboratory values for patients 1 and 2 are presented in Tables 1 and 2.

Biochemical identification and susceptibility testing

Subsequent growth on 5% sheep blood agar revealed similar morphology for all three isolates. The Microscan system identified all three isolates as *Y. enterocolitica* group. The extended biochemical profiles are shown in Table 3. There were no differences among the three isolates biochemically.

The susceptibility patterns are shown in Table 4. All three isolates were susceptible to ciprofloxacin, ticarcillin K/clavulanate, ofloxacin, cefuroxime, ampicillin/sulbactam, cefotetan, ceftazidime, imipenem, cefotaxime, gentamicin, tobramycin and trimethoprim sulfamethoxazole. They were all resistant to ampicillin, cephalothin and cefazolin.

Differences in susceptibility were seen with the following antibiotics: aztreonam, mezlocillin and piperacillin. Isolate 1 was sensitive to aztreonam (MIC = 8), while isolates 2 and 3 showed intermediate susceptibility (MIC = 16) (Table 5). Isolate 1 was sensitive to mezlocillin (MIC = 8) while isolates 2 and 3 exhibited intermediate sensitivity

Values	Patient no. 1	Patient no. 2	Normal value (1 month)*
White-blood cell count	6000	16 400	10 800 mm ³
Differential count:			
Segmented neutrophils	8	9	—
Band forms	15	28	14·10 + 4·63%
Lymphocytes	65	34	56%
Monocytes	6	21	7%
Eosinophils	1	—	3%
Atypical lymphocytes	5	6	0–3%
Metamyelocytes	—	2	11·34 + 3·59%
Platelet	3 720 000	2 560 000	300–400 × 10 ⁹ l ⁻¹
Haemoglobin	9·5	9·1	14 g dl ⁻¹ mean
Haematocrit	28%	26%	43% mean

Table 1 Laboratory values for blood obtained from patients 1 and 2

* Wintrobe's Clinical Hematology, Richard *et al.* (1993).

Table 2 Cerebrospinal fluid values for patients 1 and 2*

	Patient no. 1	Normal values†
Red blood cells	270 000	0 > 3 months
White blood cells	50 (monocytes)	0–5 > 3 months
Glucose	47 mg dl ⁻¹	50–80 mg dl ⁻¹
Protein	1435 mg dl ⁻¹	20–65 mg dl ⁻¹ (1–6 months)

* CSF culture only on patient no. 2.

† *Handbook of Paediatrics*, Merenstein *et al.* (1997).

(MIC = 32). Isolates 1 and 2 were both susceptible to piperacillin (MIC = <8 for isolate 1 and MIC = 16 for isolate 2), whereas isolate 3 showed intermediate susceptibility (MIC = 32).

Fingerprinting

RAPD-PCR using primer 1 as well as primer 4 revealed two different fingerprints among the three isolates. The banding patterns observed for *Y. enterocolitica* isolates 2 and 3 were identical when comparing the presence or absence of major bands. However, *Y. enterocolitica* isolate 1 showed a

distinctive banding pattern when compared with isolates 2 and 3. Notable pattern differences could be observed at 1 kb with primer 1 and at approximately 0.5 kb for primer 4 (Fig. 1).

DISCUSSION

Infections due to *Y. enterocolitica* have increased over the past decade and are associated with a wide variety of symptoms. Clinical *Y. enterocolitica* infections result after ingestion of the organism in contaminated food (Black *et al.* 1978) or water (Keet 1974), or by other more direct inoculation such as blood transfusions (Stenhouse and Milner 1982). While the most common presentation in children is enterocolitis with fever and diarrhoea, bacteremia can often result, as seen in this case. At least one study has shown clinical *Y. enterocolitica* to be more likely to occur in blacks and in children (Lee *et al.* 1991). African-American twin infants, who may have been somewhat immunocompromised because of their young age and low birth weight, were presented with bacteremia. Invasive yersiniosis is still an uncommon event but certain groups are at increased risk. Patients with iron overload can develop splenic or liver

Table 3 Biochemical identification of the *Yersinia enterocolitica* isolates

Biochemical profiles	Patient no. 1 Isolate 1	Isolate 2	Patient no. 2 Isolate 3
Glucose	+	+	+
Sucrose	–	–	–
Sorbitol	+	+	+
Rafinnose	–	–	–
Rhamnose	–	–	–
Arabinose	–	–	–
Inositol	–	–	–
Adonitol	–	–	–
Melibiose	–	–	–
Urea	–	–	–
Hydrogen sulphide	–	–	–
Indole	–	–	–
Lysine	–	–	–
Arginine	–	–	–
Ornithine	+	+	+
Tryptophan Deaminase	–	–	–
Esculin	–	–	–
Voges-Proskauer	–	–	–
Citrate	–	–	–
Malonate	–	–	–
o-Nitrophenyl-β, d-galactopyranoside	–	–	–
Colistin 4 µg ml ⁻¹	–	–	–
Cephalothin 8 µg ml ⁻¹	+	+	+
Oxidase	–	–	–
Identification	<i>Yersinia enterocolitica</i> group	<i>Yersinia enterocolitica</i> group	<i>Yersinia enterocolitica</i> group

Antimicrobials	Patient no. 1 Isolate 1	Isolate 2	Patient no. #2 Isolate 3
Aztreonam	S* (MIC < 8)	I (MIC = 16)	I (MIC = 16)
Ampicillin	R (MIC > 16)	R (MIC > 16)	R (MIC > 16)
Cephalothin	R (MIC > 16)	R (MIC > 16)	R (MIC > 16)
Mezlocillin	S (MIC < 8)	I (MIC = 32)	I (MIC = 32)
Ciprofloxacin	S (MIC < 1)	S (MIC < 1)	S (MIC < 1)
Piperacillin	S (MIC < 8)	S (MIC = 16)	I (MIC = 32)
Ticarcillin K Clavulanate	S (MIC < 8)	S (MIC < 8)	S (MIC < 8)
Ofloxacin	S (MIC < 2)	S (MIC < 2)	S (MIC < 2)
Cefazolin	R (MIC > 16)	R (MIC > 16)	R (MIC > 16)
Cefuroxime	S (MIC < 4)	S (MIC < 4)	S (MIC < 4)
Ampicillin/Sulbactam	S (MIC < 8)	S (MIC < 8)	S (MIC < 8)
Cefotetan	S (MIC < 4)	S (MIC < 4)	S (MIC < 4)
Ceftazidime	S (MIC < 2)	S (MIC < 2)	S (MIC < 2)
Imipenem	S (MIC < 4)	S (MIC < 4)	S (MIC < 4)
Cefotaxime	S (MIC < 4)	S (MIC < 4)	S (MIC < 4)
Gentamicin	S (MIC < 1)	S (MIC < 1)	S (MIC < 1)
Tobramycin	S (MIC < 2)	S (MIC < 2)	S (MIC < 2)
Trimethoprim/ Sulfamethoxazole	S (MIC < 2)	S (MIC < 2)	S (MIC < 2)

Table 4 Antimicrobial susceptibility patterns of the isolates

*S = sensitive; R = resistant; I = intermediate.

abscesses, and black infants will have enteritis and bacteremia. One possible explanation for an increase in the frequency of *Y. enterocolitica* infections observed among blacks during the winter is the increase in consumption of chitterlings, a popular meal of the black population during Christmas (Lee *et al.* 1990; Stoddard *et al.* 1994; Kellogg *et al.* 1995).

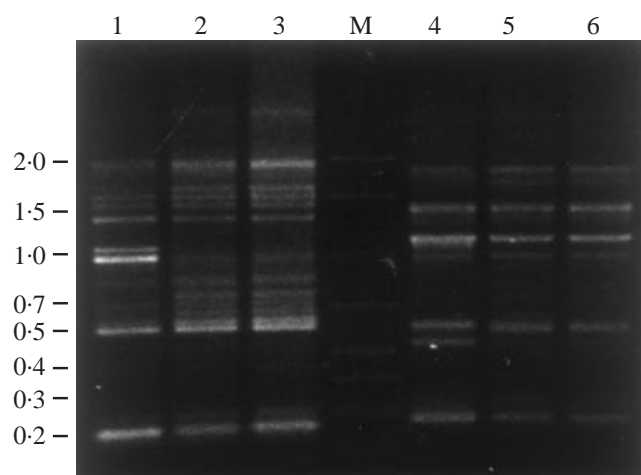


Fig. 1 RAPD analysis of *Yersinia enterocolitica* clinical isolates. Molecular size marker (Lane M). Lanes 1–3: primer 1; lanes 4–6: primer 4. *Yersinia enterocolitica* isolate 1 (lanes 1 and 4); *Y. enterocolitica* isolate 2 (lanes 2 and 5); *Y. enterocolitica* isolate 3 (lanes 3 and 6)

Numerous different typing methods have been used to distinguish different *Y. enterocolitica* strains. Biotyping and serotyping have been successfully used in some studies (Wauters *et al.* 1987), but each has been found to be less discriminatory than genotyping methods (Odinot *et al.* 1995). Isolates obtained from patients within exposure clusters have been shown to have identical biotypes and serotypes, while genotyping methods indicated that different strains were present (Odinot *et al.* 1995). Upon extended antibiogram typing, some differences were found between the isolates. Isolate 1 differed from isolates 2 and 3 in that it was susceptible to mezlocillin and aztreonam. Interestingly, isolate 3 differed from isolates 1 and 2 in that it showed intermediate susceptibility to piperacillin. However, the MIC values of piperacillin against isolates 2 and 3 were actually closer (1 well difference) than either isolate 2 or 3 compared with isolate 1 (> 1 well difference). This discrepancy is within the normal error rate for MIC assays.

Studies on genotyping methods for *Y. enterocolitica* have shown that RAPD fingerprinting offers some advantages over other methods. Plasmid analysis of *Y. enterocolitica* has not been useful because of a lack of variability in the single plasmid which exists in pathogenic strains (Nesbakken *et al.* 1987; Fukushima *et al.* 1993). Restriction endonuclease analysis of genomic DNA from *Y. enterocolitica* is discriminating, but it produces a very complex fingerprint which is difficult to interpret. Ribotyping has also been shown to be valuable, but this technique is laborious and requires extensive DNA preparation (Blumberg *et al.* 1991). Therefore, the RAPD technique has been an important method for

fingerprinting *Y. enterocolitica*. However, a common problem which exists with the RAPD technique is intra- and inter-laboratory variation in fingerprints. Due to the low stringency annealing temperatures used in the RAPD assay, it is not uncommon to find variation in the assay between users. However, recent studies by Grundman *et al.* (1997) and Gray *et al.* (1998) have found that much of the variability in RAPD fingerprints can be alleviated by using standardized reagents. Utilizing the standardized Ready-To-Go RAPD analysis beads, a *Y. enterocolitica* RAPD fingerprinting assay was developed which is rapid, requires minimal labour and is reliable and reproducible between users. The discriminatory value of the assay is demonstrated in finding *Y. enterocolitica* co-infection in one of the two twin infants described here.

Previous studies on *Y. enterocolitica* have shown that it is common to isolate different genotypes of the organism from the same patient in successive samples obtained 10 days to 2 months apart (Odinot *et al.* 1995). Although the isolates had the same bio- and serotypes, the data suggest that they were infected with different strains. Two hypotheses have arisen from these types of data: one, that a patient is colonized with several strains and that over time, one or the other is selected (Odinot *et al.* 1995). Alternatively, as a strain colonizes, the host pathogen interactions which occur induce minor genetic variation in the isolate over time, as has been described for *Legionella* spp. (Van Belkum 1994). The present studies indicate that an individual patient can be initially colonized by more than one isolate of *Y. enterocolitica*, and in patients which probably share a common route of exposure, some may not become colonized by multiple isolates. Interestingly, in the case described here, both isolates from patient 1 were obtained from blood culture, indicating that both are invasive and that there was no competitive exclusion by one isolate over another. While this case does not provide data to suggest whether multiple isolate colonization is the norm, it does provide evidence that multiple *Y. enterocolitica* isolates can and will colonize and invade in a single patient.

Obviously, this type of co-infection with multiple strains has implications for treatment and prognosis. While diagnosis of *Y. enterocolitica* may be correct, basing the treatment on *in vitro* susceptibility of one strain may be inadequate for the other isolate involved.

It may be concluded from these studies that PCR-based DNA fingerprinting of *Y. enterocolitica* has developed into a rapid, reliable technique using standardized reagents. The data from these and other studies (Odinot *et al.* 1995) indicate a potential for use in epidemiological studies, as it is possible to recognize individual strains within a biotype or serotype. In addition, it has been shown that *Y. enterocolitica* strains are capable of co-infecting patients, in this case

young infants, which has potential ramifications for diagnostics, treatment and prognosis of the co-infected patient.

ACKNOWLEDGEMENTS

The authors would like to thank the Children's Hospital of The Kings Daughters, 601 Children's Lane, Norfolk, Virginia 23507, for providing the isolates and Des Moines General Hospital for biochemical identification and susceptibility testing.

REFERENCES

- AlMohsen, I., Luedtke, G. and English, B.K. (1997) Invasive infections caused by *Yersinia enterocolitica* in infants. *Pediatric Infectious Disease Journal* **16**, 253–255.
- Baker, P.M. and Farmer, J.J. III (1982) New bacteriophage typing system for *Yersinia enterocolitica*, *Yersinia kristensenii*, *Yersinia frederiksenii*, and *Yersinia intermedia*: correlation with serotyping, biotyping, and antibiotype susceptibility. *Journal of Clinical Microbiology* **15**, 491–502.
- Black, R.E., Jackson, R.J., Tsai, T. *et al.* (1978) Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. *New England Journal of Medicine* **298**, 76–79.
- Blumberg, H.M., Kiehlbauch, J.A. and Wachsmuth, I.K. (1991) Molecular epidemiology of *Yersinia enterocolitica* O:3 infections: use of chromosomal DNA restriction fragment length polymorphisms of rRNA genes. *Journal of Clinical Microbiology* **29**, 2368–2374.
- Bottone, E.J. (1997) *Yersinia enterocolitica*: the charisma continues. *Journal of Clinical Microbiology* **10**, 257–276.
- Centers for Disease Control and Prevention (1997) Red blood cell transfusions contaminated with *Yersinia enterocolitica*—United States, 1991–96, and initiation of a national study to detect bacteria-associated transfusion reactions. *Journal of the American Medical Association* **278**, 196–197.
- Czajka, J. and Batt, C.A. (1994) Verification of causal relationships between *Listeria monocytogenes* isolates implicated in food-borne outbreaks of *Listeriosis* by randomly amplified polymorphic DNA patterns. *Journal Clinical Microbiology* **32**, 1280–1287.
- Czajka, J., Bsat, N., Piani, M. *et al.* (1993) Differentiation of *Listeria monocytogenes* and *Listeria innocua* by 16S rDNA and intraspecies discrimination of *Listeria monocytogenes* strains by random amplified polymorphic DNA polymorphisms. *Applied and Environmental Microbiology* **59**, 304–308.
- Fukushima, H., Gomyoda, M., Aleksic and Tsubokura, M. (1993) Differentiation of *Yersinia enterocolitica* serotype O:5, 27 strains by phenotypic and molecular techniques. *Journal of Clinical Microbiology* **31**, 1672–1674.
- Gray, J.T., WaKabongo, M. and Feltman, H.A. (1998) Development of a rapid and reliable method of genomic fingerprinting for *Aeromonas* spp. Abstract no. 12491. In *Proceedings of the American Society for Microbiology General Meeting, Atlanta, GA*. ASM press, Washington DC, USA.
- Grundman, H.J., Towner, K.J., Dijkshoorn, L. *et al.* (1997) Multi-center study using standardized protocols and reagents for evaluation

- of reproducibility of PCR-based fingerprinting of *Acinetobacter* spp. *Journal of Clinical Microbiology* **35**, 3071–3077.
- Janda, J.M. and Abbott, S.L. (1994) *Yersinia enterocolitica*. *Infectious Diseases for Practicing Clinicians* **18**, 9–12.
- Kapperud, G., Nesbakken, T., Aleksic, S. and Mollaret, H.H. (1990) Comparison of restriction endonuclease analysis and phenotypic typing methods for differentiation of *Yersinia enterocolitica* isolates. *Journal of Clinical Microbiology* **28**, 1125–1131.
- Keet, E.E. (1974) *Yersinia enterocolitica* septicemia. Source of infection and incubation period identified. *New York State Journal of Medicine* **74**, 2226–2230.
- Kellogg, C.M., Tarakji, E.A., Smith, M. and Brown, P.D. (1995) Bacteremia and suppurative lymphadenitis due to *Yersinia enterocolitica* in a neutropenic patient who prepared chitterlings. *Clinical Infectious Diseases* **21**, 236–237.
- Kohl, S. (1979) *Yersinia enterocolitica* infections. *Pediatric Clinics of North America* **26**, 433–443.
- Lee, L.A., Gerber, A.R., Lonsway, D.R. et al. (1990) *Yersinia enterocolitica* O:3 infections in infants and children, associated with the household preparation of chitterlings. *New England Journal of Medicine* **322**, 984–987.
- Lee, L.A., Taylor, J., Carter, G.P. et al. (1991) *Yersinia enterocolitica* O:3 and emerging cause of pediatric gastroenteritis in the United States. *Journal of Infectious Diseases* **163**, 660–663.
- Mathews, R.C. (1993) PCR fingerprinting microbes by random amplification of polymorphic DNA. *Journal of Medical Microbiology* **39**, 161–162.
- McDonald, A., Segler, S., McNeil, M. et al. (1998) Population-based surveillance for *Yersinia enterocolitica* infection in metropolitan Atlanta. *Journal of Investigational Medicine* **46**, 62 A.
- Merenstein, G.B., Kaplan, D.W. and Rosenberg, A.A. (1997) *Handbook of Pediatrics* 18th edn. pp. 992–993. Stamford, Connecticut: Appleton & Lange.
- National Committee for Clinical Laboratory Standards (NCCLS) (1998) Table 2. MIC interpretive standards ($\mu\text{g/ml}$) of *Enterobacteriaceae*. In *NCCLS Performance Standards for Antimicrobial Susceptibility Testing; Eighth Informational (Suppl.)*. pp. 51–53. Wayne, PA: NCCLS.
- Nesbakken, T., Kapperud, G., Sorum, H. and Dommarsnes, K. (1987) Structural variability of 40–50 Mdal virulence plasmids from *Yersinia enterocolitica*. Geographical and ecological distribution of plasmid variants. *Acta Pathologica et Microbiologica Scandinavica, Section B* **95**: 167–173. PMID: 2820189; UI: 87322444.
- Odinot, P.T., Meis, J.F.G.M., van den Hurk, P.J.J.C., Hoogkamp-Korstanje, J.A.A. and Melcers, W.J.G. (1995) PCR-based DNA fingerprinting discriminates between different biotypes of *Yersinia enterocolitica*. *Contributions in Microbiology and Immunology* **13**, 93–98.
- Richard, L.G., Bithell, T.C., Foerster, J., Athens, J.W. and Lukens, J.N. eds. (1993) *Wintrobe's Clinical Hematology* 9th edn, Vol. 2. Philadelphia: Lea & Febiger.
- Stenhouse, M.A.E. and Milner, L.V. (1982) *Yersinia enterocolitica*: a hazard in blood transfusion. *Transfusion* **22**, 396–398.
- Stoddard, J.J., Wechsler, D.S., Nataro, J.P. and Casella, J.F. (1994) *Yersinia enterocolitica* infection in a patient with sickle cell disease after exposure to chitterlings. *American Journal of Pediatric Hematology and Oncology* **16**, 153–155.
- Van Belkum, A. (1994) DNA fingerprinting of medically important microorganisms by use of PCR. *Clinical Microbiology Reviews* **7**, 174–184.
- Wauters, G., Kandolo, K. and Janssens, M. (1987) Revised biogrouping scheme of *Yersinia enterocolitica*. *Contributions Microbiology and Immunology* **9**, 14–21.
- Welsh, J. and McClelland, M. (1993) Characterization of pathogenic microorganisms by genomic fingerprinting using arbitrarily primed PCR. In *Diagnostic and Molecular Biology Principles, Applications* ed. Persing, D.H., Smith, T.F., Tenover, F.C. and White, T.J. pp. 595–602. Washington, D.C.: Mayo Foundation, American Society of for Microbiology.